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Temperature responses of egg production and egg development in two species of Collembola

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With 3 figures

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1. Introduction

Much attention has been paid in entomological research to the establishment of temperature responses of insect development, mostly with the purpose of predicting seasonal occurrence in the field. Only recently, this data base has been used for comparative purposes and for detecting general patterns in the evolution of insect temperatures responses (Taylor 1981). In Collembola also, a number of authors has documented temperature responses, but only a few (e.g. Thibaud 1967), discuss these in a comparative context. The present paper will center, not on the regulating effects of temperature on collembolan populations (discussed by Grégoire-Wibo & Snider 1983), but on the evolution of different patterns of adaptation. Special attention will be given to the steepness of the response, *i.e.* the amount of acceleration in development that is achieved with increasing temperature. In the case of exponential responses, this may by expressed in the familiar Q_{10}-value; if the response is linear, steepness can be expressed in the so-called thermal constant, which equals the reciprocal of the slope of the response (van Straalen 1983).

In this paper we report on temperature dependence of egg production and egg development in two species of entomobryid collembolans, *viz. Orchesella cincta* (L.) and *Tomocerus minor* (Lubbock). Information on the ecological strategies of these species can be found in Joosse (1981). Van Straalen (1985) made a quantitative study of fertility and mortality in field populations. One of the conclusions obtained there was that the species *O. cincta* exhibited a higher fertility (as estimated from recruitment into two successive generations) than the species *T. minor*. The present results supplement the estimates from field populations with experimental data under controlled temperature conditions.

2. Methods

2.0. General remarks

Orchesella cincta and *Tomocerus minor* were collected from pine needle litter in forests near Hilversum, Noordwijk and Dronten, the Netherlands. The experimental units were circular PVC boxes (⌀ 5 cm) with a 1.5 cm layer of plaster of Paris, moistened to ensure a constant humid atmosphere. Green algae, scratched from the bark of trees, were filtered and offered as food in the form of a thick suspension on paper discs. Experiments made use of temperature-controlled cabinets with a light regime of 12L/12D. Some of the cabinets were equipped with double temperature adjustment, allowing for day-night temperature fluctuations. Also, a climatized room was used which could be programmed for smooth temperature changes. Temperature recordings at the surface of the plaster of Paris of the experimental boxes revealed that, although the cabinet air temperature fluctuated within 1 to 2 °C above and below the adjusted value, fluctuations in the boxes were reduced to ± 0.5 °C. Two series of experiments were carried out, the first on egg production, the second on clutch-size and egg development.

2.1. Experiments on egg production

Two identical experiments were performed, once in April/May, using adult field animals from the overwintering autumn generation, and once in July/August, using animals mainly belonging to the generation hatched in spring. After collection from the field, animals were climatized for one week

at the experimental temperature. Each of the two experiments involved a total of 160 boxes, each containing 10 adult individuals. No discrimination of sexes was made, so that the sex ratio of each box was unknown. Boxes were randomly assigned to species *(O. cincta/T. minor)* and to temperature (5, 10, 15, 20 °C). In this manner, 20 replications per combination were obtained. For a period of six weeks in each box the number of eggs was counted. Food was refreshed every week.

The results were analysed as a completely randomized factorial design by means of analysis of variance on the number of eggs laid in 6 weeks in a box, with factors: species (2 levels), season (2 levels) and temperature (4 levels). Homogeneity of variances was judged on Bartlett's χ^2-statistic (SOKAL & ROHLF 1969), and joint normality was tested using Wilk & Shapiro's G-statistic (WILK & SHAPIRO 1968). Several transformations of the data were analysed for minimizing heterogeneity and nonnormality; of these, the square root transformation ($\sqrt{x + 1/2}$) was found to be optimal.

From the 3,200 individuals involved in these experiments a total of 398 died during the observational period (6 weeks), which gives an overall mortality rate of 12 %. No correction was made for this, nor were dead individuals replaced.

2.2. Experiments on clutch-size and egg development

To obtain egg-laying pairs, two springtails were placed together at room temperature and observed regularly. When egg-laying was noted, the pair was transferred to a fresh box and placed in one of five cabinets adjusted to constant temperatures of 10, 12, 15, 17or 20 °C. These pairs were observed daily for moulting and egg laying. The number of eggs produced between two successive moults is referred to as a clutch. At 20 °C a clutch is usually laid on one day, but at lower temperatures laying may take several days. All clutches were laid and hatched at the experimental temperatures. Development time was calculated as the difference between mean laying date and mean hatching date: mean laying and hatching dates were calculated after weighing the dates with the number of eggs laid or hatched. A total of 97 clutches has been observed in this manner.

In another experiment the effects of fluctuating temperatures on egg development were investigated. Springtails were kept 10 per box and observed daily for laying and hatching of eggs. Eggs were subjected to one of three temperature regimes: (a) constant at ± 15 °C, (b) fluctuating between 20 °C at 13.00 h and 10 °C at 1.00 h in a smooth manner (sinus regime), (c) fluctuating between 20 °C at days and 10 °C at nights, with sharp transitions at 8.00 h and 20.00 h (block regime). In each case the actual temperature in the experimental boxes was recorded continuously and the average temperature was computed from hourly readings.

3. Results

Analysis of variance applied to the results on egg production revealed highly significant species-temperature interaction, but no significant season-temperature or season-species interaction (Table 1). The temperature response of *Orchesella cincta* differs from *Tomocerus minor*, but these responses do not differ appreciably between the two successive generations. Fig. 1 gives a more detailed illustration of this result. At 5 °C *T. minor* lays more eggs than *O. cincta*, but at higher temperatures *O. cincta* is far more fertile than *T. minor*. Judged from the steepness of the temperature response, *O. cincta* is more sensitive, and reacts more strongly to increased temperatures, than *T. minor*.

Table 1. Analysis of variance on egg production. $\sqrt{x + 1/2}$ transformed data

Source of variation	df	mean square	F
species	1	2,276.485	69.856***
season	1	6.189	0.190 n.s.
temperature	3	4,553.795	139.736***
species × season	1	4.230	0.130 n.s.
species × temperature	3	882.398	27.077***
season × temperature	3	80.944	2.484 n.s.
species × season × temperature	3	11.491	0.353 n.s.
error	304	32.588	
total	**319**		

n.s. not significant, *** $p < 0.001$

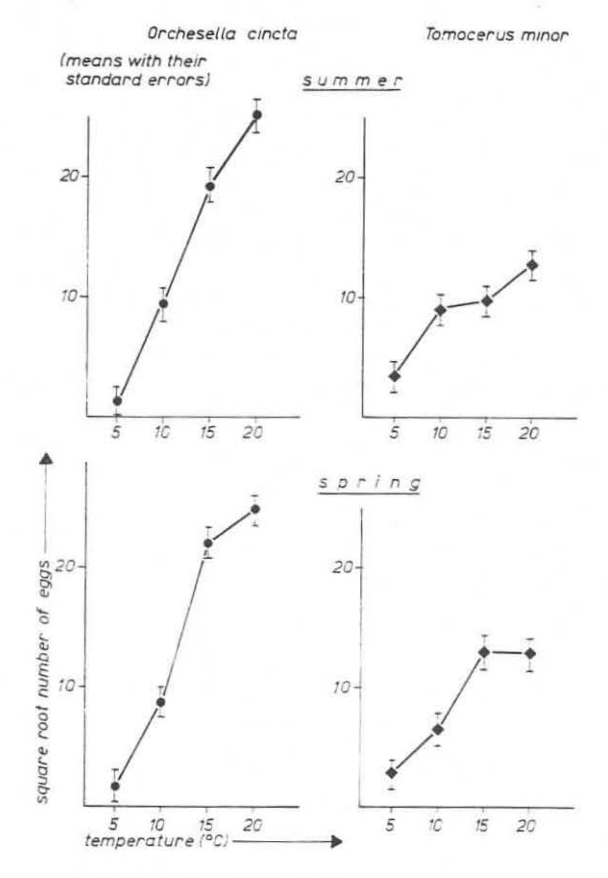


Fig. 1. Mean number of eggs laid per box (10 individuals) over 6 weeks, as a function of temperature. Each mean is based on 20 replications (boxes). Standard errors for means are derived from the analysis of variance error mean square (Table 1). All data $\sqrt{x + 1/2}$ transformed. **Summer:** adults collected in summer, belonging mainly to generation hatched in spring. **Spring:** adults collected in spring, belonging mainly to generation hatched in autumn.

Table 2. Comparison of fertility at 15 °C computed from the present experiments and from field population changes

species	field	experiments
O. cincta	19.1 11.7—26.6	14.4 11.9—17.0
T. minor	4.8 3.2—6.3	4.3 3.0—5.8

Fertility is expressed as eggs per female per week. Below each mean a 95% confidence interval is given. The first column reproduces results from demographic analysis of populations in a pine forest floor (VAN STRAALEN 1985). In this study, time was transformed such that, with respect to moulting frequency, a constant temperature of 15 °C was created. Confidence limits in this column are derived from sampling error by means of the jack-knife technique. The second column was computed from the present results at 15 °C (averaged over two seasons). Means and confidence limits given here, are back-transforms of means and confidence limits of the $\sqrt{x + 1/2}$ transformed experimental data.

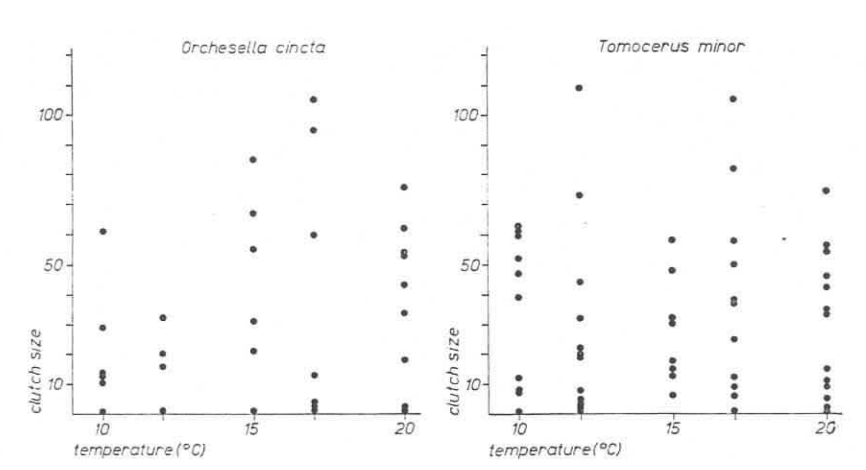


Fig. 2. Size of clutches laid at five experimental temperatures, by isolated pairs of *O. cincta* and *T. minor*.

No simple mathematical expression can describe the temperature response of egg production. Extrapolated from Fig. 1, the zero point for egg production would be 2—3 °C for *T. minor*, and 3—4 °C for *O. cincta*. Above these temperatures, the response is curved upwards (note that Fig. 1 has a square root squale), but it levels off between 15 and 20 °C. The optimum seems to lie somewhat above 20 °C in the case of *O. cincta*, and somewhat below 20 °C in the case of *T. minor* (Fig. 1). The general shape resembles curves generated by the model of SHARPE & DEMICHELE (1977); fitting of this model would require data at more temperatures.

From the eggs laid at 15 °C average fertility (eggs per female per week) was calculated by dividing the total number of eggs laid by 6 (nr. of weeks) and by 5 (presumed nr. of females per box). This fertility can be compared to fertility estimates for the same species obtained from changes in population structure in the field (VAN STRAALEN 1985). (Since 15 °C was the calibration temperature of the physiological time-scale in the field study, this comparison is relevant only at 15 °C.) A striking agreement appears between these data (Table 2). Taking confidence limits into account, there is no significant difference between fertility estimates from laboratory experiments and from demographic analysis of field populations.

Experiments on clutch-sizes revealed extreme variability (Fig. 2). Clutches may vary from 1 to more than 100 eggs. No effects of temperature, nor of species could be detected. It may be concluded that the lower fertility of *T. minor* at temperatures above 5 °C, as appearing from the preceding experiment, is not due to a lower average clutch-size.

Table 3 shows data on the viability of these clutches. If eggs did not hatch, they invariably did not develop at all ,*i.e.*, they did not show the typical split of the chorion. Usually they eventually disintegrated from fungal infection. No indications of diapausing eggs have been

Table 3. Viability of eggs as a function of clutch-size

range of clutch-sizes	*O. cincta*			*T. minor*		
	laid	hatched	survival	laid	hatched	survival
≤10	19	9	0.47	102	7	0.07
11—49	247	194	0.79	759	476	0.63
≥50	713	520	0.73	955	753	0.79
Total	**979**	**723**	**0.74**	**1816**	**1236**	**0.68**

For each range of clutch-sizes the total number of eggs laid in clutches of that range and the total number of eggs hatched is given. Overall survivorship is (979 + 1816)/(723 + 1236) = 0.70.

Table 4. Regression analysis for egg development rate on temperature

source of variation	*Orchesella cincta*			*Tomocerus minor*		
	df	mean square	F	df	mean square	F
among temperatures	4	0.013485	73.69***	4	0.005665	171.66***
linear regression	1	0.051558	64.93**	1	0.022570	752.33***
deviations from regression	3	0.000794	4.34*	3	0.000030	0.91 n.s.
within temperatures	21	0.000183		32	0.000033	
total	25			36		

n.s. not significant; * $0.01 < p < 0.05$; *** $p < 0.001$

Table 5. Estimated zero development temperatures and thermal constants for egg development

parameter	*O. cincta*	*T. minor*	units
T_0	8.6	6.9	°C
C	73.8	148.5	°C × days

T_0 = temperature below which no development occurs; C = degree-days above T_0 during development. $T_0 = -a_0/a_1$; $C = 1/a_1$; for a_0, a_1 see Fig. 3.

Table 6. Durations of egg development (in days) under fluctuating temperature regimes

temperature regime	mean temperature (°C)	*O. cincta*		*T. minor*	
		expected	observed	expected	observed
constant	14.5	12.4	12.8	19.6	19.2
sinus	15.5	10.6	10.7	17.4	16.5
block	14.5	12.4	13.3	19.6	19.6

Expected durations were computed from the mean temperature and the relationship obtained from the preceding experiment (Fig. 3).

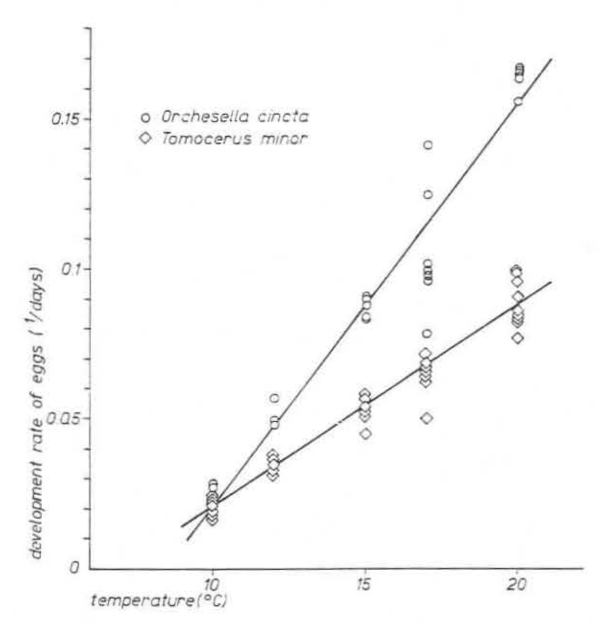


Fig. 3. Development rate of eggs (reciprocal of duration) as a function of temperature for *O. cincta* and *T. minor*. Each point gives a value for an individual clutch. Straight lines are based on regression analysis (Table 4). Estimated coefficients are: *O. cincta*: $a_0 = -0.1160$, $a_1 = 0.01355$; *T. minor*: $a_0 = -0.04676$, $a_1 = 0.006735$.

observed in these species. Eggs that did develop, hatched for almost 100%. Viability was shown to depend on clutch-size (Table 3). Especially in *T. minor*, clutches smaller than 10 eggs almost never developed. The percentage of eggs laid in small clutches is 6% for *T. minor* but only 2% for *O. cincta* (Table 3). This suggests that laying unviable eggs is more common in *T. minor*, than in *O. cincta*. The overall viability, however, does not differ significantly between the species (judged on the G-test for independence, SOKAL & ROHLF 1969), and is estimated as 70% (Table 3). Analysis not shown here indicated that viability does not change with temperature also.

Development rate of clutches was analysed by regression on temperature (Table 4). Significant variation among temperatures is explained by linear regression. In the case of *T. minor* unexplained variation is not significant: a straight line gives a correct description of the response. In the case of *O. cincta*, unexplained variation is significant at the 5% level (Table 4); inspection of the data (Fig. 3) does not, however, suggest curvilinearity of the type that is expected on biological grounds (i.e. acceleration at intermediate temperatures and levelling-off at higher temperatures). We interpret these deviations as variation due to technical imperfections, such as uncontrolled temperature changes during incubation, and we retain the linear relationship.

From least-square estimates for coefficients, zero development temperatures and thermal constants were calculated (Table 5). It appears that egg development is extremely sensitive to temperature. No development at all occurs below 7 °C, although this is a quite normal temperatures range in the Netherlands (about 40% of weekly means in a year). Even at 10 °C, development is rather slow, taking about 50 days for both species (Fig. 3). At 20 °C, however, development takes only 6 days for *O. cincta* and 11 days for *T. minor*. The sensitivity of *O. cincta* to temperature appears from its small thermal constant, when compared to *T. minor* (Table 5).

Experiments using fluctuating temperature regimes did not detect appreciable effects of fluctuations (Table 6). In each case, development could be predicted reasonably from the average temperature. Development duration was also analysed for correlation with clutch-size at constant temperature; this in no case produced significant results.

4. Discussion

4.1. Fertility and clutch-size

This study has documented a large difference in fertility (average nr. of eggs per female per week) between *Orchesella cincta* and *Tomocerus minor* at temperatures above 5 °C. Moreover, fertility observed under experimental conditions at 15 °C showed good quantitative agreement with fertility as estimated independently from population structure in the field (VAN STRAALEN 1985). Apparently, this type of experiments can provide good predictions of reproductive performance under field conditions. Extrapolation of laboratory to results fields situations is a crucial aspect in many ecological studies; in the present work, extrapolation seems to be legitimate.

Although *O. cincta* was (overall) more fertile, this fertility cannot be attributed to a higher physiological potential for reproduction. In fact, *T. minor* can lay clutches as large as *O. cincta* (Fig. 2), and the average clutch-size does not differ (see also TESTERINK 1982). The somewhat slower moulting cycle of *T. minor* (JOOSSE & VELTKAMP 1970) cannot explain quantitatively its inferior reproductive performance. The difference must be due to unsuccessful sperm transfer, such that available females of *T. minor* are not always fertilized when receptive. This can also be concluded from the observation that small clutches (≤ 10), laid by *T. minor*, almost exclusively are non-viable (Table 3). It is conceivable that these small clutches are remnants of unfertilized batches, which for the greater part are resorbed in the oviduct before laying. Similar observations were made by SNIDER (1983) for *Onychiurus folsomi*: although isolated females were capable of laying 20—40 eggs per week, egg production in cultures was much lower, showing that the full reproductive potential is not always realized.

Intraspecific communication in Collembola is achieved by means of pheromones, which may aid to synchronize moulting (LEINAAS 1983), or to increase male spermatophore deposition behaviour (WALDORF 1974). VERHOEF (1984) convincingly demonstrated that the response of reproduction efficiency to pheromone concentration is more pronounced in *O. cincta* than in *T. minor*. This makes it very likely that the observed difference in egg production is due to a more efficient pheromone mediated sperm transfer in *O. cincta*. Another argument may be derived from the occurrence of sexual dimorphism in *O. cincta* (males have distinctive colouring on antennae and abdomen, especially in reproductive instars). Communication between sexes may have led to intersexual selection, which can explain the evolution of sexual dimorphism (FISHER 1958). The presence of sexual dimorphism in *O. cincta*, and its absence in *T. minor*, suggest that communication between sexes is more developed in the former species.

4.2. Temperature responses

Responses of egg production and egg development were different. Egg production is more sensitive to temperature, as it shows a more or less exponential increase between 5 and 15 °C, while egg development responds in a linear fashion. Egg production seemed to level-off between 15 and 20 °C (Fig. 1). This may relate to the differential effects of temperature on males and females, as studied for *O. cincta* by JOOSSE *et al.* (1973). While females can increase their reproductive output by speeding up their moulting cycle with temperature (clutch-size remains the same), males produce the same number of spermatophores per time unit, and therefore their production per instar decreases with temperature. In this manner, availability of spermatophores might limit production of eggs at high temperatures.

Both species are able to produce some eggs at 5 °C, but these eggs will not develop at this temperature, since appreciable development is observed only from 10 °C upwards (*cf.* Figs. 1 and 3). This implicates that seasonal temperature changes in the field will restrict hatching of eggs to spring and summer, even if eggs are laid throughout the year (*cf.* HALE 1980 and GRÉGOIRE-WIBO 1976). The thermal constant for egg development, estimated here for *O. cincta* as 73.8 degree-days, is lower than those given for the same species by MERTENS & BLANCQUAERT (1980), who report a value of 125 degree-days, and by LINDENMANN (1950), from whose data a value of 122 degree-days can be calculated. This may indicate geographic variation in temperature sensitivity.

The ecological relevance of temperature responses in Collembola can be understood in the light of the habitat use of different species. Numerous data on vertical distribution, drought resistance and metabolic rate prove that, in its forest floor habitat, *O. cincta* has a more superficial and active way of life than *T. minor* (JOOSSE 1981, VERHOEF 1984, VAN DER WOUDE in prep.). This dichotomy is here seen to correlate with sensitivity to temperature: *O. cincta* is, both in egg production and in egg development, more sensitive than *T. minor* (*cf.* Figs. 1 and 3). With due care, this trend may be generalized for all Collembola. A review of available literature data on collembolan egg development (VAN STRAALEN in prep.) reveals that species with surface-living habits (as indicated by their life-form) tend to have steeper responses than species living deeper in the soil. Several authors (LEBRUN & VAN RUYMBEKE 1971; JOHNSON & WELLINGTON 1980) have recognized that temperature sensitivity (i.e. high Q_{10}-value or low thermal constant) will enable species to take maximal profit from even small temperature elevations in the field. Such properties seem to be more valuable for superficially living species than for deeper living species, since temperature fluctuations are more pronounced at the surface.

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In a series of experiments under controlled temperature conditions, egg production and egg development of *Orchesella cincta* (L.) and *Tomocerus minor* (LUBBOCK) has been studied. The temperature responses of egg production are non-linear, showing strong acceleration between 5 and 15 °C. The responses of egg development rate are linear up to 20 °C. No effects of fluctuating temperature regimes on egg development have been found. When kept in groups of ten individuals, *O. cincta* produces more eggs than *T. minor*, at temperatures above 5 °C. Mean clutch-size of individual females, however, does not vary with temperature and is the same for both species. It is concluded that egg production is higher for *O. cincta*, not because of a higher physiological potential, but because of a more efficient pheromone mediated sperm transfer. Fertility estimates from these experiments show good quantitative agreement with estimates obtained from mathematical analysis of changes in population structure of field populations. Temperature sensitivity is measured by the steepness of the temperature response. Both in egg production and in egg development, *O. cincta* is more sensitive to temperature than *T. minor*. In combination with evidence on the habitat use of these species, this suggests that temperature sensitivity must be interpreted as an adaptation to life at the soil surface, whereas relative temperature indifference would be more common among true soil-dwellers.
Key words: Collembola, temperature responses, reproduction, egg development, viability, fertility, thermal constant.